ON THE RELATION BETWEEN GROWTH AND MORPHOGENESIS IN THE SLIME MOLD DICTYOSTELIUM DISCOIDEUM [1]

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In the normal course of cellular slime mold development, the myxamoebae pass through the exponential growth phase and, upon its cessation, aggregate in multicellular masses. The number of aggregative centers formed has been shown to be a function of the number of cells and of the population density (Sussman and Noel, 1952). From these and other relationships (Sussman, 1952) it can be inferred that each aggregate requires for its formation the presence of a special cell called an initiator, to which neighboring cells respond, and only a small proportion of the population can so serve under the conditions employed. In *Dictyostelium discoideum* wild type, the ratio of the initiator to responder cells has been found to be 1:2200. That the system is under genetic control is shown by the fact that mutants of *D. discoideum*, as well as wild type representatives of other species, display significantly different ratios (Sussman and Noel, 1952; Sussman, 1955).

In the past, Raper (1940) has presented evidence to indicate that aggregation can begin only when growth ceases and that the aggregation of a stationary phase population can be prevented and indeed reversed by further additions of nutrient material. The present study demonstrates an extension of this conclusion: that is, that cells taken from any phase of the growth cycle, including newly germinated spores, can aggregate when further growth is prevented and, in fact, give quantitatively the same aggregative performance as do stationary phase myxamoebae.

Methods

A. Organisms and media. The organism used in this study is representative of *D. discoideum* Raper, strain NC-4. Stock cultures were grown on glucose-peptone agar in association with *A. aerogenes* by methods previously described (Sussman, 1951). Under these conditions the myxamoebae exhaust the bacterial supply after about 44 hours incubation at 22° C. and aggregate and construct fruits in the ensuing 24 hours. Spore suspensions from these fruits were used as inocula for special growth experiments to be described in the next section.

B. Aggregation experiments. All the methods and procedures employed have been described in detail elsewhere (Sussman and Noel, 1952). Washed myxamoebae were suspended in salt solution (Bonner, 1947), counted with a hemocytometer, and dispensed in drops on a washed agar-distilled water substratum. The excess fluid was absorbed by the agar leaving the cells randomly distributed within the confines of the drop. Under these conditions the amoebae remained constant in number, aggregated in normal fashion after 12 hours and produced

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typical fruits. Control of the number of myxamoebae delivered was achieved by appropriate dilution of the original cell suspensions. The population density of the cells on the agar was determined by the volume of the drop delivered.

Results

A. The growth curve of D. discoideum on complete and restricted media

Spores, taken from a 4–6 day stock plate, were suspended in salt solution and spread on glucose peptone agar with 0.1 cc. of a 48-hour broth culture of *A. aerogenes*. The initial spore count per plate was 1×10^6. At intervals, duplicate plates were harvested using a finger and 10 cc. distilled water delivered in two batches. Control experiments using known numbers of cells indicated a recovery in excess of 95% of the cells on the plates. Replicate counts with the hemocytometer were made of each harvest. Figure 1 shows the results of two experiments (upper curve).

A technical difficulty precluded the use of this system for studying the aggrega-

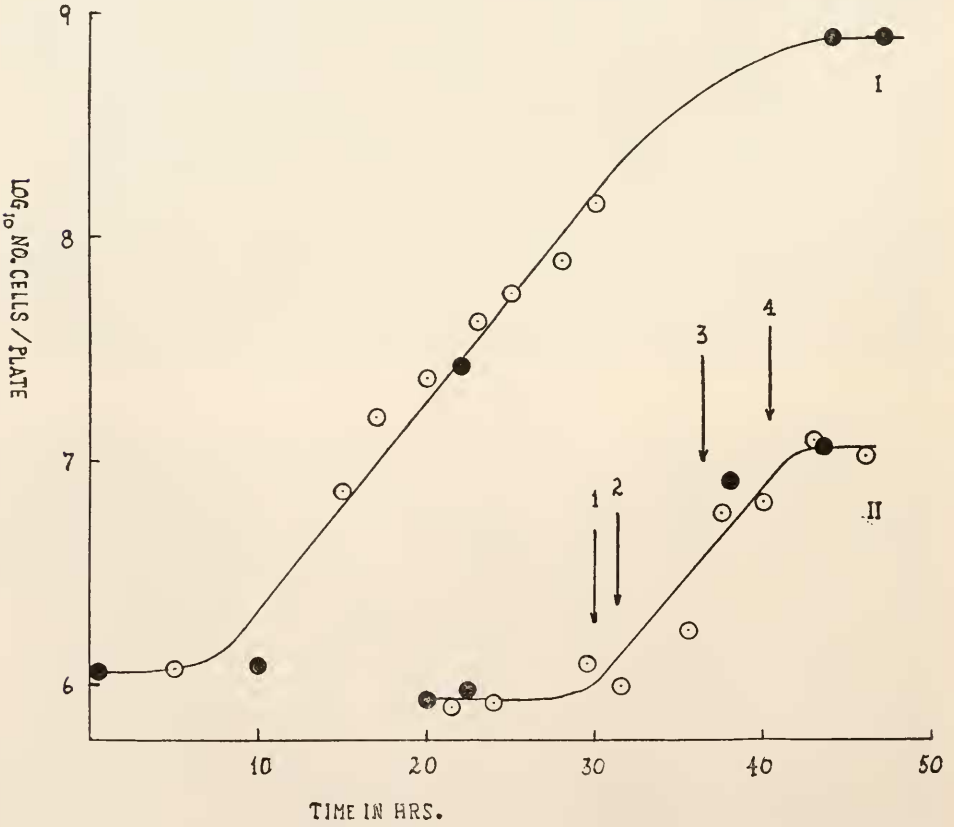

Figure 1. Growth curves of *D. discoideum* on complete (I) and restricted (II) media. The arrows indicate the times at which cell samples were taken for tests of aggregative performance.

tive performances of cells in different growth phases. During the log phase the number of bacteria was so great that clean preparations could not be obtained even by exhaustive washing in the centrifuge. The delivery of such contaminated suspensions to washed agar plates for the aggregation experiments led to considerable growth of the myxamoebae and rendered the results meaningless.

To avoid this hindrance, a restricted medium was used on which the bacteria remained in more convenient balance with the myxamoebae. A 48-hour broth culture of *A. aerogenes* was washed once in the centrifuge and suspended in water to ½ the original volume. One-half cc. aliquots were spread on washed agar plates with the spore inoculum and incubated at 22° C. Figure 1 (lower curve) shows the growth curve obtained from two experiments. The arrows show where samples of cells were taken for aggregation experiments to be described. The data reveal only minor differences in the growth curves on the two types of media. The lag phase, including the germination period, was about 7 hours on complete medium and about 11 hours on restricted. True exponential increases occurred on both media, the generation times being approximately the same (ca. three hours).

The stationary phase was attained at about 40 hours on complete medium and at 24 hours on restricted medium. A population of nearly 10^9 myxamoebae per plate on the former indicated a total of 9.5 generations of growth. On the latter medium the stationary population was slightly more than 10^7 per plate, the result of 3.8 generations of growth.

B. Aggregative performance of cells in different growth phases

As mentioned previously the number of centers of aggregation formed by a population of myxamoebae is a function of the number of cells and of the population density. The curve in Figure 2 demonstrates these relationships for samples of 10,000 washed myxamoebae taken from the stationary growth phase on glucose-peptone agar plates and dispensed on washed agar over a range of population densities. It is seen that at densities below 80 cells per mm.^{2} no centers appeared after 24 hours' incubation period. At high densities center formation was suppressed to some extent and at the optimal density, around 200 cells per mm.^{2}, a maximal number of centers was formed. The distribution of initiator cells to responder cells thus shown is 1:2100, in very close agreement with previous evaluations (Sussman and Noel, 1952). To determine the aggregative performances of cells in different growth phases, cultures grown on restricted medium were harvested at stages shown by the arrows in Figure 1. These stages were: 1 and 2, newly germinated myxamoebae at the end of the lag phase and/or at the beginning of the log phase; 3, at mid-log phase; 4, at the end of the log phase. The cells were washed by repeated centrifugation, suspended in salt solution, and replicate samples of 10,000 cells were delivered to washed agar over a range of population densities. It should be noted that even after thorough washing, the cells were contaminated to a slight extent with occluded bacteria. To avoid growth of the bacteria and therefore of the amoeba on the aggregation plates, streptomycin sulphate (Nutritional Biochemical) was added to the agar in final concentration of 0.5 gm. per liter. Unpublished experiments have shown that streptomycin prevents growth of the myxamoebae under these conditions but does not interfere in any observable manner with aggregation or fruit construction.

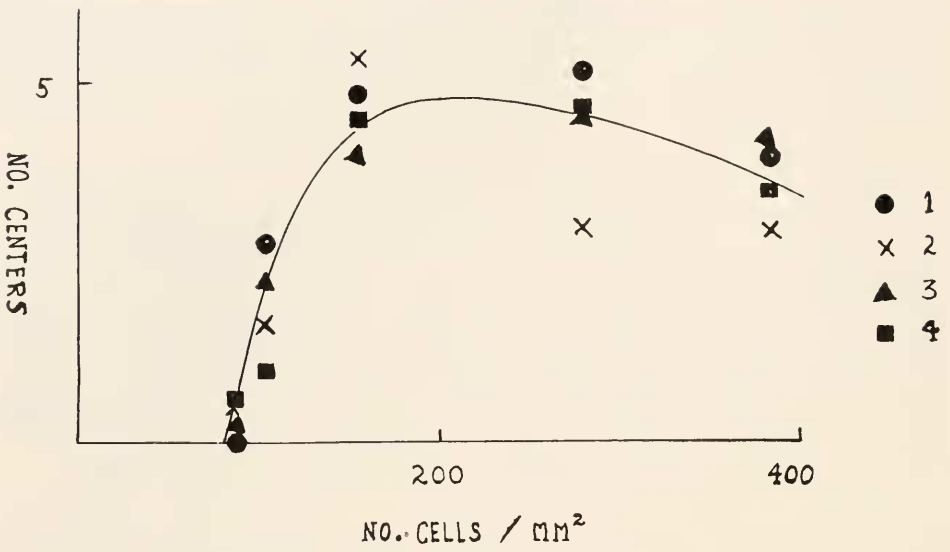

FIGURE 2. Relation between center formation and population density. Cell samples 1–4 were taken at times indicated by the arrows in Figure 1 and were washed by centrifugation and suspended in salt solution. Aliquots containing 10,000 cells were dispensed on washed agar over a range of densities. The densities were checked by direct observation with an ocular grid. The number of aggregative centers were counted after 24 hours. The ordinate shows the number of centers per aliquot.

A single experiment was performed at each of the stages mentioned. Figure 2 summarizes the data, each point representing the mean of at least six replicate samples. Aliquots containing relatively small numbers of cells (10,000) had to be used because of the practical limits imposed by the growth stages at which the cells were harvested. This magnified the random sampling error. Nevertheless, the points bracket the normal curve quite closely, and it is clear that no significant difference is apparent between the aggregative performance of cells in lag and log phases and that of cells from the stationary phase.

DISCUSSION

Evidence gained from a number of experimental directions has made it necessary to categorize aggregating myxamoebae as being either initiator cells that provide the aggregative stimulus or responder cells that answer it. One can account for this phenotypic divergence in at least two ways: a) The initiator cells arise spontaneously in the population via a stable genetic alteration. The ratio of initiator to responder cells is governed by selective pressures of the environment during the growth of the population. b) The matrix of conditions attending the cessation of growth induces a small proportion of the population to attain initiative capacity above the threshold necessary to attract responder cells under the conditions employed. This involves no prior genetic alteration but merely results from the exploitation of random physiological differences among the cells at that time. Thus, prior to the stoppage of growth, there are no initiator and responder cells *per se*.

The experiments described here support the second of these alternatives. In the mature fruiting body the spores represent a non-random sample of the original myxamoeboid population. This follows from the fact that the fates of the myxamoebae (whether they are to become spores, stalk cells, or basal disk cells) are determined by the order in which they entered the aggregates. The prestalk cells enter first, prespore cells next, and prebasal disk cells last (Bonner, 1944). Were the difference between initiator and responder cell due to a persistent, spontaneous, genetic change, one would expect that a newly germinated spore population should display a skewed distribution of initiator cells and that, during the subsequent growth of these cells, selective conditions would systematically alter the distribution until the customary ratio of 1:2200 was regained. In contrast, the second alternative does not carry with it the necessity of presuming these fluctuations in the initiator: responder ratio during the growth cycle. If the passage from a stage of active growth and synthesis to the stationary state itself evoked the random physiological variation that in turn determined whether a cell was to be an initiator or responder, then the distribution of initiators might be the same no matter what the past history of the cells.

The view is in accord with previously published results (Sussman, 1952, 1955) that indicate a spectrum of initiative capacity in the population. Thus in any system, the number of centers actually formed would depend both upon this spectrum and upon the level of sensitivity of the responder cells.

Summary

1. The growth kinetics of *Dictyostelium discoideum* have been determined on complete and restricted media.

2. Myxamoebae were taken from different parts of the lag and log phases and allowed to aggregate under conditions permitting quantitative examination. No difference could be discerned between the aggregative performance of these cells and that of stationary phase myxamoebae with respect to the number of centers formed or the population density response.

3. The bearing of these results upon the mechanisms of cellular differentiation during slime mold development is discussed.

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